American Journal of Hematology 62:103–105 (1999)

Clinical Analysis and TPO Levels in Three Patients With Refractory Thrombocytopenia

Katsunori Kyoda,[1]* Shinobu Nakamura,[2] Minoru Takeshima,[2] Hirokazu Okumura,[2] Shigeki Ohtake,[2] Tamotsu Matsuda,[2] and Hisatsugu Miyakoshi[1]

[1]The 1st of Internal Medicine, Koseiren Takaoka Hospital, Takaoka City, Toyama, Japan
[2]Third Department of Internal Medicine, Kanazawa University School of Medicine, Kanazawa City, Ishikawa, Japan

Refractory thrombocytopenia (RTC) is a counter-concept to refractory anemia, which is characterized by isolated thrombocytopenia associated with clonal chromosomal abnormality. The diagnosis of RTC is difficult to establish based on morphologic features alone. And steroid therapy for RTC is often ineffective. We examined 3 patients with RTC to identify its characteristics and measured serum thrombopoietin levels. The mean platelet count was 5.1 × 10^4/μl and the mean age was 64 years. None of our patients had clinical nor laboratory evidence of liver dysfunction, renal disease or disseminated intravascular coagulation. All patients were negative for antiplatelet antibody, PA-IgG and anticardiolipin-β2GPI antibody. Leukocyte alkaline phosphatase level was low in two patients. Clonal chromosomal abnormalities of different types were detected in all patients. Bone marrow smears showed micromegakaryocytes. But there were no apparent morphological abnormalities of erythroid and granuloid series. Thrombopoietin levels, as determined by enzyme-linked immunosorbent assay, varied from <0.2 to 1.40 fmol/ml. We could not find the screening tool of RTC. In conclusion, there is a need to identify RTC from isolated thrombocytopenia because the patients with RTC don't have good prognosis as patients with isolated thrombocytopenia. Cytogenetic analysis is necessary to establish the diagnosis of RTC. We recommend that a patient above 50 years of age presenting with isolated thrombocytopenia and a low leukocyte alkaline phosphatase score should be suspected of having RTC. Am. J. Hematol. 62:103–105, 1999.

Key words: MDS; clonal chromosomal abnormality; refractory thrombocytopenia

INTRODUCTION

Myelodysplastic syndrome (MDS) constitutes a heterogeneous collection of acquired clonal disorders of hematopoiesis. The most prominent manifestations are cytopenias related to progressive bone marrow failure.

Anemia is the most common cytopenia in MDS and refractory anemia is the largest (40–46%) category of MDS [1,2]. But isolated thrombocytopenia is rare in MDS [3–7]. Some case reports of MDS with isolated thrombocytopenia have been previously described, but in most of them diagnosis was established based on morphologic features of peripheral blood and bone marrow cells [3–5]. Menke et al. [6] analyzed 354 cases of MDS associated with clonal chromosomal abnormalities and identified 11 cases of isolated thrombocytopenia. They named this condition "refractory thrombocytopenia (RTC)", as a counter-concept to refractory anemia.

The diagnosis of RTC is difficult to establish based on morphologic features alone, because dysplasia may not always be detectable. So cytogenetics have been analyzed necessarily in patients with unexplained isolated thrombocytopenia in our hospital, and we experienced three patients with RTC. We examined three patients to identify the characteristics of RTC and measured their serum thrombopoietin (TPO) levels.

PATIENTS AND METHODS

All patients diagnosed as having isolated thrombocytopenia associated with clonal chromosomal abnormali-

*Correspondence to: Katsunori Kyoda, The 1st of Internal Medicine, Koseiren Takaoka Hospital, 5-10, Eiraku-cho, Takaoka City, Toyama, Japan.

Received for publication 27 July 1998; Accepted 2 June 1999

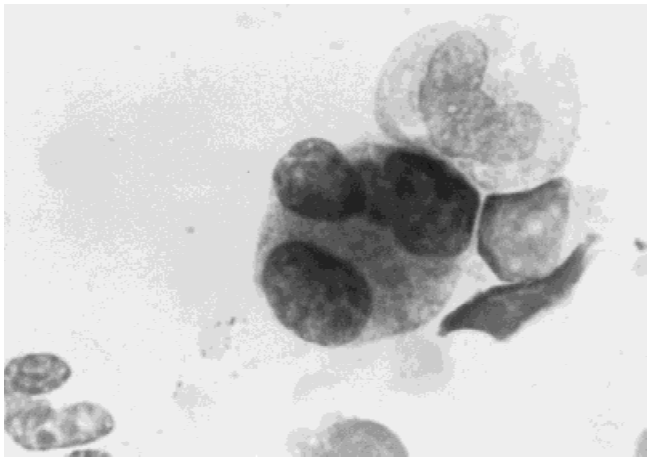

Fig. 1. Bone marrow smears of three patients with RTC showed the presence of micromegakaryocytes.

ties were clinically evaluated. None of them had anemia nor granulocytopenia, hepatosplenomegaly, laboratory evidence of liver dysfunction, renal disease or disseminated intravascular coagulation. All patients had normal levels of serum iron, Vitamin B12 and follic acid, and they were negative for antiplatelet antibody, PA-IgG and anticardiolipin-β2GPI antibody. Bone marrow smears of all patients showed the presence of micromegakaryocytes (Fig. 1). There were no apparent morphological abnormalities of the erythroid and granuloid cell series. Leukocyte alkaline phosphatase (LAP) stain in peripheral neutrophils was performed using the fast blue RR staining method [8]. Scoring of LAP positive cells was done according to Kaplow's method [9]. Serum TPO levels were determined with an enxyme-linked immunosorbent assay, which uses a mouse monoclonal antibody directed against recombinant human TPO [10]. None of them had received specific therapeutic agents prior to the study.

CASE REPORTS (TABLE I)

Patient 1 was a 57-year-old man who presented in December 1990 with examination of chest abnormal shadow. He had 4,100/μl white blood cells (WBC), 14.4 g/dl hemoglobin (Hb) and 6.0×10^4/μl platelet count (PLT). Bone marrow cellularity was slightly decreased. The quantity of megakaryocyte was slightly decreased. Cytogenetic study disclosed the existence of chromosomal abnormality 46 XY, 20 q^- cocomitant with a normal karyotype.

Patient 2 was a 61-year-old woman who visited with Raynaud's sign in March 1993. She had 4,300/μl WBC, 12.6 g/dl Hb, and 4.5×10^4/μl PLT. The level of LAP score was low. Bone marrow cellularity was normal. The quantity of megakaryocyte was slightly increased. Cytogenetic analysis revealed an abnormality of 46 XX, inv.(2)(p11;q21) for all 20 analysed cells.

Patient 3 was 73-year-old man who presented in October 1997 with examination of preoperation. He had 4,100/ml WBC, 13.8 g/dl Hb and 4.9×10^4/μl PLT. The level of LAP score was low. Bone marrow cellularity was normal. The quantity of megakaryocyte was normal. Cytogenetic study disclosed the existence of chromosome abnormality 46 XY, del(20)(q11) cocomitant with a normal karyotype.

TPO levels of three RTC patients were within normal range (Table I). All patients were symptom-free and required no medication. No additional cytopenia developed in these three patients during follow up (mean 25 months, range 6–36 months).

DISCUSSION

RTC is a rare type of MDS. Menke et al. [6] reported that RTC accounted for only 0.9% of the patients with MDS. They stated that diagnosis of RTC was difficult despite careful morphologic examinations of peripheral blood and bone marrow aspirates, because dysplasia may not always be detectable. It is important to distinguish RTC from isolated thrombocytopenia (ITP) cases as soon as possible before having the results of cytogenetic analyses, because steroid therapy for RTC is, reportedly, often ineffective. We think the guideline of treatment for RTC should be the same as those of MDS.

We tried to identify the characteristics of RTC in these three patients and found two points in common. Firstly, none of the patients was not young (mean 64 years) similar to past reports [5,6]. Secondly, two patients had a low LAP score. A low LAP score is frequently recognized in MDS [9,10]. This phenomenon seems to support the concept that RTC is a type of MDS.

Clonal cytogenetic abnormalities have been observed in 33–79% of patients with MDS [11–13]. According to the past reports, cytogenetic abnormalities of chromosome 3 or 5, monosomy or deletion of chromosome 20 (20 q^-) and trisomy 8 have been reported to be common in patients with RTC. Two patients in this series had deletion of chromosome 20, which is the same as those of patients described in past reports. It is reported that 20q^- was represented about 2% of de novo MDS [16]. And the patients with 20 q^- had a tendency towards lower incidence of anemia.

As for TPO levels, Kunishima et al. [17] reported that TPO levels may distinguish MDS from ITP patients. In our study, the TPO levels of patients with RTC were within normal range and not higher than those of patients with ITP (data not shown). In our series only the TPO level seems to be indistinguishable RTC from ITP cases without other data. Further study is necessary to understand the meaning of TPO levels in RTC.

TABLE I. Clinical and Laboratory Characteristics of Three Patients*

Case	Age	WBC	Hb	Plt	LAP score	Serum TPO	Karyotype [number of metaphases]
1	57	4,100/μl	14.4 g/dl	6.0×10^4/μl	277	<0.20 fmol/ml	46 XY,20q$^-$ [13]/46 XY [7]
2	61	4,300/μl	12.6 g/dl	4.5×10^4/μl	155	0.74 fmol/ml	46 XX,inv.(2)(p11;q21) [20]
3	73	4,100/μl	13.8 g/dl	4.9×10^4/μl	24	1.40 fmol/ml	46 XY,del(20)(q11) [15]/46 XY [5]

*LAP, leukocyte alkaline phosphatase; TPO, thrombopoietin; inv, inversion; del, deletion.

In the study by Menke et al. [6] additional cytopenias developed in 64% of 11 patients with RTC, and 45% of 11 patients in whom the condition progressed to acute myelogenous leukemia during follow up (mean 30 months). Our patients have not developed additional cytopenias as yet. We think we should continue to carefully follow up our patients, because they don't have good prognosis as patients with ITP. We tried to identify the screening tools of RTC, but every clinical data without cytogeneticus in this study were not enough to diagnosis the patients as having RTC.

In conclusion, cytogenetic analysis is necessary to establish the diagnosis of isolated thrombocytopenia, even if the patient is suspected of having ITP. Cytogenetic study is too expensive to examine routinely in patients with unexplained isolated thrombocytopenia. We recommend that a patient above 50 years of age presenting with isolated thrombocytopenia and a low LAP score should be suspected of having RTC. Clonal chromosomal abnormalities are needed to diagnosis as having RTC. But if there were patients with RTC without clonal chromosomal abnormalities, they would probably be misdiagnosed as having ITP.

ACKNOWLEDGMENTS

We thank Pharmaceutical Development Labolatory, Kirin Brewery Co. Ltd, for referring specimens and clinical information.

REFFERENCES

1. Toyama K, Ohyashiki K, Yoshida Y, Abe T, Asano S, Hirai H, Hirashima K, Hotta T, Kuramoto A, Kuriya S, Miyazaki T, Kakishita E, Mizoguchi H, Okada M, Shirakawa S, Takaku F, Tomonaga M, Uchino H, Yasunaga K, Nomura T. Clinical implications of chromosomal abnormalities in 401 patients with myelodysplastic syndromes: a multicentric study in Japan. Leukemia 7:499–508, 1993.
2. Oguma S, Yoshida Y, Uchino H, Maekawa T, Nomura T, Mizoguchi H and the Refractory Anemia Study Group of the Ministry of Health and Welfare. Clinical characteristics of Japanese patients with primary myelodysplastic syndromes: a co-operative study based on 838 cases. Leuk Res 19:219–225, 1995.
3. Tricot C, Criel A, Verwilghen RL. Thrombocytopenia as presenting symptom of preleukemia in 3 patients. Scand J Haematol 28:243–250, 1982.
4. Mitsutani S, Kohriyama T, Kamio M, Saitoh T. Isolated thrombocytopenia as presenting syndromes of MDS. Jpn J Clin Hematol 28:945–950, 1987.
5. Najean Y, Lecompte T. Chronic pure thrombocytopenia in elderly patients: an aspect of the myelodysplastic syndrome. Cancer 64:2506–2510, 1989.
6. Menke DM, Colon-Otero G, Cockerill KJ, Jenkins RB, Noel P, Pierre RV. Refractory thrombocytopenia: a myelodysplastic syndrome that may mimic immune thrombocytopenic purpura. Am J Clin Pathol 98:502–510, 1992.
7. Imoto S, Hayashi Y, Matsui T, Murayama T, Iwata N, Matsuoka H, Nagata A, Itoh H, Chihara K. Refractory thrombocytopenia with chromosome 11q23 abnormality. Ann Hematol 73:99–102, 1996.
8. Tomonaga M, Sasaki T, Okuzaki M. Studies on alkaline phosphatase. I. Use of naphthol AS-MX phosphate; fast blue RR staining method. Acta Hematol Jpn 26:179–192, 1963.
9. Kaplow LS. Cytochemistry of leukocyte alkaline phosphatase. Am J Clin Pathol 39:439, 1963.
10. Tahara T, Usuki K, Sato H, Ohashi H, Morita H, Tsumura H, Matsumoto A, Miyazaki H, Urabe A, Kato T. A sensitive sandwich ELISA for measuring thrombopoietin in human serum: serum thrombopoietin levels in healthy volunteers and in patients with haemopoietic disorders. Br J Haematol 93:783–788, 1996.
11. Bendix-Hansen K, Bergmann OJ. Evaluation of neutrophil alkaline phosphatase (NAP) activity in untreated myeloproliferative syndromes and in leukemoid reactions. Scad J Haematol 35:219–224, 1985.
12. Bendix-Hansen K, Kerndrup G. Myeloperoxidase-deficient polymorphonuclear leukocytes. (V): relation to FAB classification and neutrophil alkaline phosphatase activity in primary myelodysplastic syndromes. Scand J Haematol 35:197–200, 1985.
13. Yunis JJ, Rydell RE, Oken MM, Arnesen MA, Mayer MG, Lobell M. Refined chromosome analysis as an independent prognostic indicator in de novo myelodysplastic syndromes. Blood 67:1721–1730, 1986.
14. Morel P, Hebbar M, Lai JL, Duhamel A, Preudhomme C, Wattel E, Bauters F, Fenaux P. Cytogenetic analysis has strong independent prognostic value in de novo myelodysplastic syndromes and can be incorporated in a new scoring system: a report on 408 cases. Leukemia 7:1315–1323, 1993.
15. Haase D, Fonatsch C, Freund M, Wormann B, Bodenstein H, Bartels H, Stollmann-Gibbels B, Lengfelder E. Cytogenetic findings in 179 patients with myelodysplastic syndromes. Ann Hematol 70:171–187, 1995.
16. Wattel E, Lai JL, Hebbar M, Preudhomme C, Grahek D, Morel P, Bauters F, Fenaux P. De novo myelodysplastic syndrome (MDS) with deletion of the long arm of chromosome 20: a subtype of MDS with distinct hematological and prognostic features? Leuk Res 17:921–926, 1993.
17. Kunishima S, Tahara T, Kato T, Kobayashi S, Saito H, Naoe T. Serum thrombopoietin and plasma glycocalicin concentrations as useful diagnostic markers in thrombocytopenic disorders. Eur J Haematol 57: 68–71, 1996.